

## EP 1 131 447 B1

Glu Gly Tyr Gln Ser Ser Gly Thr Ser Ser Ile Asn Val Gly Gly  
 180 185 190

5

&lt;210&gt; 11

&lt;211&gt; 101

&lt;212&gt; PRT

&lt;213&gt; Streptomyces lividans

10

&lt;400&gt; 11

Ala Thr Thr Ile Thr Thr Asn Gln Thr Gly Thr Asp Gly Met Tyr Tyr  
 1 5 10 15

15

Ser Phe Trp Thr Asp Gly Gly Gly Ser Val Ser Met Thr Leu Asn Gly  
 20 25 30

Gly Gly Ser Tyr Ser Thr Gln Trp Thr Asn Cys Gly Asn Phe Val Ala  
 35 40 45

20

Gly Lys Gly Trp Ser Thr Gly Asp Gly Asn Val Arg Tyr Asn Gly Tyr  
 50 55 60

25

Phe Asn Pro Val Gly Asn Gly Tyr Gly Cys Leu Tyr Gly Trp Thr Ser  
 65 70 75 80

Asn Pro Leu Val Glu Tyr Tyr Ile Val Asp Asn Trp Gly Ser Tyr Arg  
 85 90 95

30

Pro Thr Gly Thr Tyr Lys Gly Thr Val Ser Ser Asp Gly Gly Thr Tyr  
 100 105 110

Asp Ile Tyr Gln Thr Thr Arg Tyr Asn Ala Pro Ser Val Glu Gly Thr  
 115 120 125

35

Lys Thr Phe Gln Gln Tyr Trp Ser Val Arg Gln Ser Lys Val Thr Ser  
 130 135 140

Gly Ser Gly Thr Ile Thr Thr Gly Asn His Phe Asp Ala Trp Ala Arg  
 145 150 155 160

40

Ala Gly Met Asn Met Gly Gln Phe Arg Tyr Tyr Met Ile Asn Ala Thr  
 165 170 175

45

Glu Gly Tyr Gln Ser Ser Gly Ser Ser Asn Ile Thr Val Ser Gly  
 180 185 190

50

&lt;210&gt; 12

&lt;211&gt; 189

&lt;212&gt; PRT

&lt;213&gt; Streptomyces sp.

&lt;400&gt; 12

55

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Ala Thr Thr Ile Thr Asn Glu Thr Gly Tyr Asp Gly Met Tyr Tyr Ser  
 1 5 10 15  
 Phe Trp Thr Asp Gly Gly Gly Ser Val Ser Met Thr Leu Asn Gly Gly  
 20 25 30  
 Gly Ser Tyr Ser Thr Arg Trp Thr Asn Cys Gly Asn Phe Val Ala Gly  
 35 40 45  
 Lys Gly Trp Ala Asn Gly Gly Arg Arg Thr Val Arg Tyr Thr Gly Trp  
 50 55 60  
 Phe Asn Pro Ser Gly Asn Gly Tyr Gly Cys Leu Tyr Gly Trp Thr Ser  
 65 70 75 80  
 Asn Pro Leu Val Glu Tyr Tyr Ile Val Asp Asn Trp Gly Ser Tyr Arg  
 85 90 95  
 Pro Thr Gly Glu Thr Arg Gly Thr Val His Ser Asp Gly Gly Thr Tyr  
 100 105 110  
 Asp Ile Tyr Lys Thr Thr Arg Tyr Asn Ala Pro Ser Val Glu Ala Pro  
 115 120 125  
 Ala Ala Phe Asp Gln Tyr Trp Ser Val Arg Gln Ser Lys Val Thr Ser  
 130 135 140  
 Gly Thr Ile Thr Thr Gly Asn His Phe Asp Ala Trp Ala Arg Ala Gly  
 145 150 155 160  
 Met Asn Met Gly Asn Phe Arg Tyr Tyr Met Ile Asn Ala Thr Glu Gly  
 165 170 175  
 Tyr Gln Ser Ser Gly Ser Ser Thr Ile Thr Val Ser Gly  
 180 185

&lt;210&gt; 13

&lt;211&gt; 189

&lt;212&gt; PRT

&lt;213&gt; Thermomonospora fusca

&lt;400&gt; 13

Ala Val Thr Ser Asn Glu Thr Gly Tyr His Asp Gly Tyr Phe Tyr Ser  
 1 5 10 15  
 Phe Trp Thr Asp Ala Pro Gly Thr Val Ser Met Glu Leu Gly Pro Gly  
 20 25 30  
 Gly Asn Tyr Ser Thr Ser Trp Arg Asn Thr Gly Asn Phe Val Ala Gly  
 35 40 45

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Lys Gly Trp Ala Thr Gly Gly Arg Arg Thr Val Thr Tyr Ser Ala Ser  
50 55 60

5 Phe Asn Pro Ser Gly Asn Ala Tyr Leu Thr Leu Tyr Gly Trp Thr Arg  
65 70 75 80

Asn Pro Leu Val Glu Tyr Tyr Ile Val Glu Ser Trp Gly Thr Tyr Arg  
85 90 95

10 Pro Thr Gly Thr Tyr Met Gly Thr Val Thr Thr Asp Gly Gly Thr Tyr  
100 105 110

15 Asp Ile Tyr Lys Thr Thr Arg Tyr Asn Ala Pro Ser Ile Glu Gly Thr  
115 120 125

Arg Thr Phe Asp Gln Tyr Trp Ser Val Arg Gln Ser Lys Arg Thr Ser  
130 135 140

20 Gly Thr Ile Thr Ala Gly Asn His Phe Asp Ala Trp Ala Arg His Gly  
145 150 155 160

Met His Leu Gly Thr His Asp Tyr Met Ile Met Ala Thr Glu Gly Tyr  
165 170 175

25 Gln Ser Ser Gly Ser Ser Asn Val Thr Leu Gly Thr Ser  
180 185

30 <210> 14  
<211> 190  
<212> PRT  
<213> Trichoderma harzianum  
<400> 14

35 Gln Thr Ile Gly Pro Gly Thr Gly Tyr Ser Asn Gly Tyr Tyr Tyr Ser  
1 5 10 15

40 Tyr Trp Asn Asp Gly His Ala Gly Val Thr Tyr Thr Asn Gly Gly Gly  
20 25 30

Gly Ser Phe Thr Val Asn Trp Ser Asn Ser Gly Asn Phe Val Gly Gly  
35 40 45

45 Lys Gly Trp Gln Pro Gly Thr Lys Asn Lys Val Ile Asn Phe Ser Gly  
50 55 60

Ser Tyr Asn Pro Asn Gly Asn Ser Tyr Leu Ser Ile Tyr Gly Trp Ser  
65 70 75 80

50 Arg Asn Pro Leu Ile Glu Tyr Tyr Ile Val Glu Asn Phe Gly Thr Tyr  
85 90 95

55

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5 Asn Pro Ser Thr Gly Ala Thr Lys Leu Gly Glu Val Thr Ser Asp Gly  
 100 105 110  
 Ser Val Tyr Asp Ile Tyr Arg Thr Gln Arg Val Asn Gln Pro Ser Ile  
 115 120 125  
 10 Ile Gly Thr Ala Thr Phe Tyr Gln Tyr Trp Ser Val Arg Arg Asn His  
 130 135 140  
 Arg Ser Ser Gly Ser Val Asn Thr Ala Asn His Phe Asn Ala Trp Ala  
 145 150 155 160  
 15 Ser His Gly Leu Thr Leu Gly Thr Met Asp Tyr Gln Ile Val Ala Val  
 165 170 175  
 Glu Gly Tyr Phe Ser Ser Gly Ser Ala Ser Ile Thr Val Ser  
 180 185 190

&lt;210&gt; 15

&lt;211&gt; 178

&lt;212&gt; PRT

&lt;213&gt; Trichoderma reesei

&lt;400&gt; 15

30 Ala Ser Ile Asn Tyr Asp Gln Asn Tyr Gln Thr Gly Gly Gln Val Ser  
 1 5 10 15  
 Tyr Ser Pro Ser Asn Thr Gly Phe Ser Val Asn Trp Asn Thr Gln Asp  
 20 25 30  
 35 Asp Phe Val Val Gly Val Gly Trp Thr Thr Gly Ser Ser Ala Pro Ile  
 35 40 45  
 Asn Phe Gly Gly Ser Phe Ser Val Asn Ser Gly Thr Gly Leu Leu Ser  
 50 55 60  
 40 Val Tyr Gly Trp Ser Thr Asn Pro Leu Val Glu Tyr Tyr Ile Met Glu  
 65 70 75 80  
 45 Asp Asn His Asn Tyr Pro Ala Gln Gly Thr Val Lys Gly Thr Val Thr  
 85 90 95  
 Ser Asp Gly Ala Thr Tyr Thr Ile Trp Glu Asn Thr Arg Val Asn Glu  
 100 105 110  
 50 Pro Ser Ile Gln Gly Thr Ala Thr Phe Asn Gln Tyr Ile Ser Val Arg  
 115 120 125  
 Asn Ser Pro Arg Thr Ser Gly Thr Val Thr Val Gln Asn His Phe Asn  
 130 135 140  
 55

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5 Trp Ala Ser Leu Gly Leu His Leu Gly Gln Met Met Asn Tyr Gln Val  
 145 150 155 160  
 Val Ala Val Glu Gly Trp Gly Gly Ser Gly Ser Ala Ser Gln Ser Val  
 165 170 175  
 10 Ser Asn  
 <210> 18  
 <211> 190  
 <212> PRT  
 15 <213> Trichoderma reesei  
 <400> 18  
 Gln Thr Ile Gln Pro Gly Thr Gly Tyr Asn Asn Gly Tyr Phe Tyr Ser  
 1 5 10 15  
 20 Tyr Trp Asn Asp Gly His Gly Gly Val Thr Tyr Thr Asn Gly Pro Gly  
 20 25 30  
 25 Gly Gln Phe Ser Val Asn Trp Ser Asn Ser Gly Asn Phe Val Gly Gly  
 35 40 45  
 Lys Gly Trp Gln Pro Gly Thr Lys Asn Lys Val Ile Asn Phe Ser Gly  
 50 55 60  
 30 Ser Tyr Asn Pro Asn Gly Asn Ser Tyr Leu Ser Val Tyr Gly Trp Ser  
 65 70 75 80  
 Arg Asn Pro Leu Ile Glu Tyr Tyr Ile Val Glu Asn Phe Gly Thr Tyr  
 85 90 95  
 35 Asn Pro Ser Thr Gly Ala Thr Lys Leu Gly Glu Val Thr Ser Asp Gly  
 100 105 110  
 40 Ser Val Tyr Asp Ile Tyr Arg Thr Gln Arg Val Asn Gln Pro Ser Ile  
 115 120 125  
 Ile Gly Thr Ala Thr Phe Tyr Gln Tyr Trp Ser Val Arg Arg Asn His  
 130 135 140  
 45 Arg Ser Ser Gly Ser Val Asn Thr Ala Asn His Phe Asn Ala Trp Ala  
 145 150 155 160  
 Gln Gln Gly Leu Thr Leu Gly Thr Met Asp Tyr Gln Ile Val Ala Val  
 165 170 175  
 50 Glu Gly Tyr Phe Ser Ser Gly Ser Ala Ser Ile Thr Val Ser  
 180 185 190  
 55 <210> 17  
 <211> 190  
 <212> PRT

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<213> Trichoderma viride  
<400> 17

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5      Gln Thr Ile Gln Pro Gly Thr Gly Phe Asn Asn Gly Tyr Phe Tyr Ser
      1              5              10              15

      Tyr Trp Asn Asp Gly His Gly Gly Val Thr Tyr Thr Asn Gly Pro Gly
      20              25              30

10     Gly Gln Phe Ser Val Asn Trp Ser Asn Ser Gly Asn Phe Val Gly Gly
      35              40              45

      Lys Gly Trp Gln Pro Gly Thr Lys Asn Lys Val Ile Asn Phe Ser Gly
      50              55              60

15     Ser Tyr Asn Pro Asn Gly Asn Ser Tyr Leu Ser Val Tyr Gly Trp Ser
      65              70              75              80

      Arg Asn Pro Leu Ile Glu Tyr Tyr Ile Val Glu Asn Phe Gly Thr Tyr
      85              90              95

      Asn Pro Ser Thr Gly Ala Thr Lys Leu Gly Glu Val Thr Ser Asp Gly
      100             105             110

25     Ser Val Tyr Asp Ile Tyr Arg Thr Gln Arg Val Asn Gln Pro Ser Ile
      115             120             125

      Ile Gly Thr Ala Thr Phe Tyr Gln Tyr Trp Ser Val Arg Arg Thr His
      130             135             140

30     Arg Ser Ser Gly Ser Val Asn Thr Ala Asn His Phe Asn Ala Trp Ala
      145             150             155             160

      Gln Gln Gly Leu Thr Leu Gly Thr Met Asp Tyr Gln Ile Val Ala Val
      165             170             175

35     Glu Gly Tyr Phe Ser Ser Gly Ser Ala Ser Ile Thr Val Ser
      180             185             190

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40

&lt;210&gt; 18

&lt;211&gt; 586

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

45

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: TrX synthetic sequence

&lt;400&gt; 18

50

55

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ctagcttaagg aggcctgcaga tgcaaacaaat acaaccaggga accgggttaca acaacgggtta 60  
ctttctacagc tacttggaaag atggccatgg tgggtgtacc tacacaaacg ggcccggagg 120  
ccaatttagc gtcaattgggt ctaactccgg aaacttcgta ggtgggaaaag gtcggcaacc 180  
5 cgggaccaaa aacaagggtga tcaacttttc tggatcttat aatccgaatg ggaattcata 240  
ctcaagcgtc tatgggtgggt ctgaaaacc accgattgaa tattacattg tcgaaaattc 300  
cggtaacctac aatccgagta ccggcgccac aaaattaggc gaagtcacta gtgatggatc 360  
cgtatatgat atctaccgta cccaacgct taatcagcca tcgatcattg gaaccgccac 420  
cttttatcag tactggagtg cttagcgtaa tcatcgagc cccggctcgg ttaatactgc 480  
10 gaatcacttt aatgcacggg cacagcaagg gttaacccta ggtacaatgg attatcaaat 540  
cgtagcggcg gaaggctact ctctcgatgg ttccgctagt attacagtga gctaaa 596

<210> 19  
15 <211> 40  
<212> DNA  
<213> Artificial Sequence  
<220>  
<223> Description of Artificial Sequence:Trx-110C Synthetic Sequence  
20 <400> 19  
atatcggat ccatcacaag lgactcgcc taatttgg 40  
<210> 20  
<211> 68  
<212> DNA  
25 <213> Artificial Sequence  
<220>  
<223> Description of Artificial Sequence:Tx-110C-2  
<400> 20

30 ggcgcacaaa attaggcgaa gtcacttggt atggatccgt atatgatata taccgtaccc 60  
aacgcgtt 68

<210> 21  
35 <211> 42  
<212> DNA  
<213> Artificial Sequence  
<220>  
<223> Description of Artificial Sequence:Tx-103b  
40 <400> 21  
aatcagccat cgtatcattg aaccgccacc ttatatcagt ac 42  
<210> 22  
<211> 64  
<212> DNA  
45 <213> Artificial Sequence  
<220>  
<223> Description of Artificial Sequence:XyTv-109  
Synthetic sequence  
<400> 22  
50 ggtggcgggt ccaatgatcg atggctgatt aacgcgttgg gtacggtaga tacc 54  
<210> 23  
<211> 48  
<212> DNA  
<213> Artificial Sequence  
55 <220>  
<223> Description of Artificial Sequence:Tx-108b  
<400> 23  
cgaaccggag ctccgatgat tacgtctaac atccagtagc tgataaaa 48

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<210> 24  
<211> 52  
<212> DNA  
<213> Artificial Sequence  
5 <220>  
<223> Description of Artificial Sequence:Tx-154C  
Synthetic sequence  
<400> 24  
ctagggttaa ccttgtgat gccaggcat taaagtgga tgcagtata ac 52  
10 <210> 25  
<211> 84  
<212> DNA  
<213> Artificial Sequence  
<220>  
15 <223> Description of Artificial Sequence:Tx-154C-2  
<400> 25  
  
tggagtgtca gacgtaatca tcggagctcc ggttcgggta atactgcatg ccactttaa 60  
20 gcctgggcac agcaaggggc aacc 84  
  
<210> 26  
<211> 34  
<212> DNA  
25 <213> Artificial Sequence  
<220>  
<223> Description of Artificial Sequence:Tx-162H-3  
<400> 26  
ccactcaat gcatgggca agcacgggtt aacc 34  
30 <210> 27  
<211> 42  
<212> DNA  
<213> Artificial Sequence  
<220>  
35 <223> Description of Artificial Sequence:TrX-162H-4  
<400> 27  
ctagggttaa ccgtgctgt gccatgcat tgaagtgga tg 42  
<210> 28  
<211> 58  
40 <212> DNA  
<213> Artificial Sequence  
<220>  
<223> Description of Artificial Sequence:XyTv-101  
<400> 28  
45 tcgacaatt cgtaccta aatccagca ccggcgccac aaaattaggc gaagtcac 58  
<210> 29  
<211> 52  
<212> DNA  
<213> Artificial Sequence  
<220>  
50 <223> Description of Artificial Sequence :XyTv-102  
<400> 29  
tagtgatgga tccgtatag atatctaccg taccacacgc gttaatcagc ca 52  
<210> 30  
55 <211> 60  
<212> DNA  
<213> Artificial Sequence  
<220>



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<223> Description of Artificial Sequence:TrX-103  
<400> 30  
tcgatcatg gaaccgccac ctttatcag tactggagtg ttagacgtaa lcatcggagc 60  
<210> 31  
6 <211> 69  
<212> DNA  
<213> Artificial Sequence  
<220>  
10 <223> Description of Artificial Sequence:XyTv-104  
<400> 31  
  
tccgggtcgg ttaatactgc gaatcacttt aatgcattggg cacagcaagg gttaacccta 60  
ggtacaatg 69  
15  
<210> 32  
<211> 67  
<212> DNA  
<213> Artificial Sequence  
20 <220>  
<223> Description of Artificial Sequence:XyTv-105  
<400> 32  
  
gattatcaaa tcgtacgggt ggaaggctac ttctcagatg gtcccgctag tattacagtg 60  
agctaaa 67  
25  
  
<210> 33  
<211> 53  
30 <212> DNA  
<213> Artificial Sequence  
<220>  
<223> Description of Artificial Sequence:XyTv-106 synthetic sequence  
<400> 33  
35 gatcttago tcactgaat actagcggaa ccactcgaga agtagcctc cac 53  
<210> 34  
<211> 66  
<212> DNA  
<213> Artificial Sequence  
40 <220>  
<223> Description of Artificial Sequence:XyTv-107  
<400> 34  
  
cgctacgatt tgataatcca ttgtacctag ggctaaccct tgctgtgccc atgcattaaa 60  
gtgatt 66  
45  
  
<210> 35  
<211> 60  
50 <212> DNA  
<213> Artificial Sequence.  
<220>  
<223> Description of Artificial Sequence:TrX-108  
<400> 35  
55 cgcagtalta accgaaccgg agctccgatg attacgcta acactccagt actgataaaa 60  
<210> 36  
<211> 73  
<212> DNA

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&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence:XyTv-110

&lt;400&gt; 36

5 atatacggat ccatcactag tgacttcgcc taattttgtg gcgcgggtac tggattgta 60  
ggtaaccgaaa ctg 73

&lt;210&gt; 37

&lt;211&gt; 76

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence:TrX-1

&lt;400&gt; 37

10 ctagctaagg aggctgcaga tgcaaacat acaaccagga accggttaca acaacggta 60  
ctttacagc tattgg 76

20

&lt;210&gt; 38

&lt;211&gt; 78

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence:XyTv-2

&lt;400&gt; 38

25 aacgatggcc atggtgggtg taccratata aacgggcccg gaggccaatt tagcgtcaat 60  
tggcttaact ccggaaac 78

&lt;210&gt; 39

&lt;211&gt; 78

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence:TrX-3

&lt;400&gt; 39

35 ttctgtaggtg gaaaagggtg gcaaccggg accaanaata aggtgatcaa ctctctctgga 60  
tcttataatc cgaatggg 78

45

&lt;210&gt; 40

&lt;211&gt; 74

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence:XyTv-4

&lt;400&gt; 40

50 aattcact taagcgtcta cggctgggtc agaaaccac tgattgaata ttacattgtc 60  
gaaaattctg gtac 74

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<210> 41  
<211> 85  
<212> DNA  
<213> Artificial Sequence  
5 <220>  
<223> Description of Artificial Sequence :XyTv-5  
<400> 41  
gcaaattttc gacaaatgtaa tattcaatca gtgggtttct agaccagcca tagacgctta 60  
10 agtatgaatt cccattcgga ttata 85  
<210> 42  
<211> 78  
15 <212> DNA  
<213> Artificial Sequence  
<220>  
<223> Description of Artificial Sequence:Trx-6Synthetic sequence  
<400> 42  
20 agatccagag aagttgatca ccttatcttc ggtcccgggt tgccaacctt tccacctac 60  
gaagtttccg gaggtaga 78  
<210> 43  
<211> 84  
<212> DNA  
<213> Artificial Sequence  
25 <220>  
<223> Description of Artificial Sequence:XyTv-7  
Synthetic sequence  
30 <400> 43  
ccaattgacg cttaaattggc ctccggggccc gtttgatat gtaacaccac catggccatc 60  
35 gtccaatag ctgtaaaagt aacc 84  
<210> 44  
40 <211> 51  
<212> DNA  
<213> Artificial Sequence  
<220>  
<223> Description of Artificial Sequence:TrX-8 synthetic  
45 sequence  
<400> 44  
gttggtgtaa ccggttcctg gttgtattgt ttgcatctgc agcctccta g 51  
<210> 45  
<211> 40  
50 <212> DNA  
<213> Artificial Sequence  
<220>  
<223> Description of Artificial Sequence:Tx-108C  
synthetic sequence  
55 <400> 45  
atatacggat ccatcactcg tgcattcgcc taatttttg 40  
<210> 48  
<211> 68

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<212> DNA  
<213> Artificial Sequence  
<220>  
<223> Description of Artificial Sequence:Tx-108C-2  
5 <400> 48  
gcccacaaa attagggcga tgcactagtg atggatccgt atatgatatt taccgtaccc 60  
aacgcgtt 68

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<210> 47  
<211> 52  
<212> DNA  
<213> Artificial Sequence  
<220>  
15 <223> Description of Artificial Sequence:Tx-158C-162H  
synthetic sequence  
<400> 47  
ctaggggttaa cccgtgtgat gccagcaat taaagtatt tgcagtatta ac 52  
20 <210> 48  
<211> 84  
<212> DNA  
<213> Artificial Sequence  
<220>  
25 <223> Description of Artificial Sequence:TX-158C-162H-2  
<400> 48  
tggagtgtta gacgtaatca tcggagctcc ggttcgggta atactgcxaa tcaacttcaat 60  
30 tgcctgggcac agcacgggtt aacc 84

<210> 49  
<211> 40  
<212> DNA  
35 <213> Artificial Sequence  
<220>  
<223> Description of Artificial Sequence:Tx-108c-110c  
synthetic sequence  
<400> 49  
40 atatacggat ccatcacaag tgcattcgcc taattttgtg 40  
<210> 50  
<211> 68  
<212> DNA  
<213> Artificial Sequence  
45 <220>  
<223> Description of Artificial Sequence:Tx-108C-110C-2  
synthetic sequence  
<400> 50  
50 gcccacaaa attagggcga tgcacttctg atggatccgt atatgatatt taccgtaccc 60  
aacgcgtt 68

55  
<210> 51  
<211> 52  
<212> DNA  
<213> Artificial Sequence  
<220>

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<223> Description of Artificial  
Sequence:Tx-154C-155C-152H synthetic sequence

<400> 51  
ctagggttaa cccgtgtgat gccagcaat taaagtggca tgcagatta ac 52

<210> 52

<211> 84

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial

Sequence:Tx-154C-158C-162H-2

<400> 52

tgagtggtta gacgtaatca tccgagctcc ggttcgggta atactgcacg ccactttaat 60  
tgctgggcac agcacgggtt aacc 84

<210> 53

<211> 190

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: TrX amino acid  
sequence

<400> 53.

Gln Thr Ile Gln Pro Gly Thr Gly Tyr Asn Asn Gly Tyr Phe Tyr Ser  
1 5 10 15

Tyr Trp Asn Asp Gly His Gly Gly Val Thr Tyr Thr Asn Gly Pro Gly  
20 25 30

Gly Gln Phe Ser Val Asn Trp Ser Asn Ser Gly Asn Phe Val Gly Gly  
35 40 45

Lys Gly Trp Gln Pro Gly Thr Lys Asn Lys Val Ile Asn Phe Ser Gly

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50 55 60

5 Ser Tyr Asn Pro Asn Gly Asn Ser Tyr Leu Ser Val Tyr Gly Trp Ser  
65 70 75 80

Arg Asn Pro Leu Ile Glu Tyr Tyr Ile Val Glu Asn Phe Gly Thr Tyr  
85 90 95

10 Asn Pro Ser Thr Gly Ala Thr Lys Leu Gly Glu Val Thr Ser Asp Gly  
100 105 110

15 Ser Val Tyr Asp Ile Tyr Arg Thr Gln Arg Val Asn Gln Pro Ser Ile  
115 120 125

Ile Gly Thr Ala Thr Phe Tyr Gln Tyr Trp Ser Val Arg Arg Asn His  
130 135 140

20 Arg Ser Ser Gly Ser Val Asn Thr Ala Asn His Phe Asn Ala Trp Ala  
145 150 155 160

Gln Gln Gly Leu Thr Leu Gly Thr Met Asp Tyr Gln Ile Val Ala Val  
165 170 175

25 Glu Gly Tyr Phe Ser Ser Gly Ser Ala Ser Ile Thr Val Ser  
180 185 190

30 <210> 54  
<211> 198  
<212> DNA  
<213> Artificial Sequence  
<220>  
<223> Description of Artificial Sequence: TrX-DS1  
cassette  
35 <400> 54

40 gcgccacaaa attaggcgaa gtcacttctg atggatccgt atatgatcacc taccgtaccc 60  
aacgcgttaa tcagccatcg atcattggaa ccgccacett ttatcagtac tggagtgtta 120  
gacgtaatca tcggagctcc ggctcgggta atactgcatg ccactttaat gcttgggcac 180  
agcaagggtt aaccctag 198

45 <210> 55  
<211> 67  
<212> PRT  
<213> Artificial Sequence  
<220>  
<223> Description of Artificial sequence: TrX-DS1  
cassette aa  
50

55

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<400> 55  
 Gly Ala Thr Lys Leu Gly Glu Val Thr Cys Asp Gly Ser Val Tyr Asp  
           1                  5                  10                  15  
 5 Ile Tyr Arg Thr Gln Arg Val Asn Gln Pro Ser Ile Ile Gly Thr Ala  
                   20                  25                  30  
 10 Thr Phe Tyr Gln Tyr Trp Ser Val Arg Arg Asn His Arg Ser Ser Gly  
                   35                  40                  45  
 Ser Val Asn Thr Ala Cys His Phe Asn Ala Trp Ala Gln Gln Gly Leu  
                   50                  55                  60  
 15 Thr Leu Gly  
           65

<210> 56  
 <211> 15  
 <212> PRT  
 <213> Artificial Sequence  
 <220>  
 <223> Description of Artificial Sequence:TrX-162H-DS1  
 25 cassette aa  
 <400> 56

Ala Cys His Phe Asn Ala Trp Ala Gln His Gly Leu Thr Leu Gly  
           1                  5                  10                  15  
 30

<210> 57  
 <211> 198  
 <212> DNA  
 35 <213> Artificial Sequence  
 <220>  
 <223> Description of Artificial Sequence:TrX-162H-DS2  
 cassette  
 <400> 57

40 gcgccacaaa attaggcgaa tgcactagtg atggatccgt atatgatacc taccgtaccc 60  
 aacggcgtaa ccagccatcg atcattggaa ccgccaccc ttatcagtag cggagtgtta 120  
 gacgtaatca tcggagctcc ggttcgggta atactgcaaa tcactttaat tgctgggac 180  
 agcaagggtt aaccttag  
 45

<210> 58  
 <211> 67  
 <212> PRT  
 50 <213> Artificial Sequence  
 <220>  
 <223> Description of Artificial Sequence:TrX-162H-DS2  
 cassette aa  
 <400> 58

59

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Gly Ala Thr Lys Leu Gly Glu Cys Thr Ser Asp Ser Ser Val Tyr Asp  
 1 5 10 15  
 5 Ile Tyr Arg Thr Gln Arg Val Asn Gln Pro Ser Ile Ile Gly Thr Ala  
 20 25 30  
 Thr Phe Tyr Gln Tyr Trp Ser Val Arg Arg Asn His Arg Ser Ser Gly  
 35 40 45  
 10 Ser Val Asn Thr Ala Asn His Phe Asn Cys Trp Ala Gln His Gly Leu  
 50 55 60  
 Thr Leu Gly  
 65  
 15  
 <210> 59  
 <211> 198  
 <212> DNA  
 20 <213> Artificial Sequence  
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 cassette  
 <400> 59  
 25  
 gcgcacacaaa attaggcgaa tgcacttggtg atggatccgt atatgacatc taccgtaccc 60  
 aacgcgttaa tcagccatcg atcattggaa ccgccacatt ttatcagtac tggagtgtta 120  
 gacgtaatca tcggaetccc ggttcggtta atactgcatg ccactttaat tgcctggcac 180  
 30 agcacgggtt aaccctag 198

35 <210> 60  
 <211> 67  
 <212> PRT  
 <213> Artificial Sequence  
 <220>  
 <223> Description of Artificial Sequence:TrX-162H-DS4  
 cassette aa  
 40 <400> 60

Gly Ala Thr Lys Leu Gly Glu Cys Thr Cys Asp Gly Ser Val Tyr Asp  
 1 5 10 15  
 45 Ile Tyr Arg Thr Gln Arg Val Asn Gln Pro Ser Ile Ile Gly Thr Ala  
 20 25 30

50

65



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Thr Phe Tyr Gln Tyr Trp Ser Val Arg Arg Asn His Arg Ser Ser Gly  
                     35                                    40                                    45

Ser Val Asn Thr Ala Cys His Phe Asn Cys Trp Ala Gln His Gly Leu  
                     50                                    55                                    60

Thr Leu Gly  
                     65

<210> 61  
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 <223> Description of Artificial Sequence: TrX-162H-DS1  
 cassette  
 <400> 61  
 catgccactt caatgcatgg gcacagcacg ggtaacccct ag 42

## Claims

1. An isolated Family 11 xylanase comprising at least one intramolecular disulphide bond, and a basic amino acid at position 162 (*Trichoderma reesei* xylanase II numbering) or its equivalent, said position determined from sequence alignment of said isolated xylanase with said *Trichoderma reesei* xylanase II amino acid sequence defined in SEQ ID NO:16, said isolated xylanase exhibiting at least 40% of optimal activity, when compared to wild-type xylanase, from pH 3.5 to pH 6.0, and from 40 to 60°C, and at least 30% of optimal activity after a pre-incubation step for 30 minutes at 70°C, 80°C or 90°C in the presence of 40% glycerol; a pre-incubation step for 30 or 60 minutes at 62.5°C in the absence of a stabilizer or a pre-incubation step of 30 minutes at 64°C or 68°C in the absence of a stabilizer.
2. The isolated xylanase of claim 1, wherein said basic amino acid is selected from the group consisting of lysine, arginine and histidine.
3. The isolated xylanase of claim 2, wherein said basic amino acid is histidine.
4. The isolated xylanase of claim 1 comprising two disulfide bridges.
5. The isolated xylanase of claim 1 selected from the group consisting of TrX-162H-DS1, TrX-162H-DS2, TrX-162H-DS4, and TrX-DS8.
6. The isolated xylanase of claim 5, wherein said xylanase is TrX-162H-DS1.
7. The isolated xylanase of claim 5, wherein said xylanase is TrX-162H-DS2.
8. The isolated xylanase of claim 5, wherein said xylanase is TrX-162H-DS4.
9. The isolated xylanase of claim 5, wherein said xylanase is TrX-DS8.
10. A method of obtaining Family 11 xylanase comprising:
  - i) selecting an organism that expresses xylanase activity, and obtaining said xylanase from said organism;
  - ii) determining whether said xylanase exhibits at least 40% of optimal activity, when compared to wild-type xylanase, from pH 3.5 to pH 6.0, and from 40 to 60°C; and
  - iii) determining whether said xylanase is a Family 11 xylanase and exhibits at least 30% of optimal activity after

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- a pre-incubation step for 30 minutes at 70°C, 80°C or 90°C in the presence of 40% glycerol;  
 a pre-incubation step for 30 or 60 minutes at 62.5°C in the absence of a stabilizer; or  
 a pre-incubation step of 30 minutes at 64°C or 68°C in the absence of a stabilizer;  
 iv) determining whether said xylanase has a basic amino acid at position 162 (*Trichoderma reesei* xylanase II numbering), said position determined from sequence alignment of said isolated xylanase with said *Trichoderma reesei* xylanase II amino acid sequence defined in SEQ ID NO: 16, and an intra-molecular disulfide bond; and  
 v) retaining said xylanase that expresses these properties.
11. The method of claim 10, wherein step i) includes partially purifying said xylanase.
12. A method of preparing animal feed comprising applying the isolated xylanase of claim 1 onto said animal feed to produce a xylanase-animal feed combination, and heat sterilizing said xylanase-animal feed combination.
13. The method of claim 12, wherein said animal feed is a poultry or swine feed.
14. A method of preparing animal feed comprising, applying the xylanase obtained from step v) of claim 10 onto said animal feed to produce a xylanase-animal feed combination, and heat sterilizing said xylanase-animal feed combination.
15. The method of claim 14, wherein said animal feed is a poultry or swine feed.
16. The isolated xylanase of claim 1, wherein the xylanase is recombinant.
17. The isolated xylanase of claim 1 comprising one disulfide bridge.
18. The isolated xylanase of claim 1, said xylanase obtained from an organism selected from the group consisting of *Aspergillus niger*, *Aspergillus kawachii*, *Aspergillus tubingensis*, *Bacillus circulans*, *Bacillus pumilus*, *Bacillus subtilis*, *Cellulomonas fimi*, *Chainla* spp., *Clostridium acetobutylicum*, *Clostridium stercoarum*, *Fibrobacter succinogenes*, *Neosartillasterix patriciarum*, *Nocardopsis dassonvillei*, *Ruminococcus flavefaciens*, *Schizophyllum commune*, *Streptomyces lividens*, *Streptomyces* sp. No. 36a, *Streptomyces thermoviolaceus*, *Thermomonospora fusca*, *Trichoderma harzianum*, *Trichoderma reesei*, and *Trichoderma viride*.
19. An isolated Family 11 xylanase characterized in comprising at least one intramolecular disulfide bond, and a basic amino acid at position 162 (*Trichoderma reesei* xylanase II numbering) or its equivalent, said position determined from sequence alignment of said isolated xylanase with said *Trichoderma reesei* xylanase II amino acid sequence defined in SEQ ID NO:16, said xylanase exhibiting at least 30% of optimal activity, when compared to wild-type xylanase, after  
 a pre-incubation step for 30 minutes at 70°C, 80°C or 90°C in the presence of 40% glycerol;  
 a pre-incubation step for 30 or 60 minutes at 62.5°C in the absence of a stabilizer, or  
 a pre-incubation step of 30 minutes at 64°C or 68°C in the absence of a stabilizer.

## Patentansprüche

1. Isolierte Xylanase der Familie 11, umfassend mindestens eine intramolekulare Disulfid-Bindung und eine basische Aminosäure in Position 162 (*Trichoderma reesei* Xylanase II Numerierung) oder deren Äquivalent, wobei diese Position durch Sequenzvergleich der genannten isolierten Xylanase mit der Aminosäuresequenz der genannten *Trichoderma reesei* Xylanase II, wie sie in SEQ ID NO:16 definiert ist, festgelegt wurde, wobei die genannte isolierte Xylanase, im Vergleich mit Xylanase vom Wildtyp, mindestens 40% der optimalen Aktivität im Bereich von pH 3,5 bis 6,0 und von 40 bis 60°C zeigt, und nach  
 einem 30 minütigen Vorinkubations-Schritt bei 70°C, 80°C oder 90°C in der Gegenwart von 40% Glycerin,  
 einem 30- oder 60 minütigen Vorinkubations-Schritt bei 62,5°C in Abwesenheit eines Stabilisators oder  
 einem 30-minütigen Vorinkubations-Schritt bei 64°C oder 68°C in Abwesenheit eines Stabilisators  
 mindestens 30% der optimalen Aktivität zeigt.
2. Isolierte Xylanase gemäß Anspruch 1, worin die genannte basische Aminosäure ausgewählt ist aus der Gruppe, die aus Lysin, Arginin und Histidin besteht.

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3. Isolierte Xylanase gemäß Anspruch 2, worin die genannte basische Aminosäure Histidin ist.
4. Isolierte Xylanase gemäß Anspruch 1, umfassend zwei Disulfid-Brücken.
5. Isolierte Xylanase gemäß Anspruch 1, ausgewählt aus der Gruppe, die aus TrX-162H-DS1, TrX-162H-DS2, TrX-162H-DS4 und TrX-DS8 besteht.
6. Isolierte Xylanase gemäß Anspruch 5, worin die Xylanase TrX-162H-DS1 ist.
7. Isolierte Xylanase gemäß Anspruch 5, worin die Xylanase TrX-162H-DS2 ist.
8. Isolierte Xylanase gemäß Anspruch 5, worin die Xylanase TrX-162H-DS4 ist.
9. Isolierte Xylanase gemäß Anspruch 5, worin die Xylanase TrX-DS8 ist.
10. Verfahren zum Gewinnen einer Xylanase der Familie 11, umfassen:
  - i) Auswählen eines Organismus', der Xylanase-Aktivität exprimiert, und Gewinnen der Xylanase aus diesem Organismus;
  - ii) Feststellen, ob diese Xylanase, im Vergleich mit Xylanase vom Wildtyp, mindestens 40% der optimalen Aktivität im Bereich von pH 3,5 bis pH 6,0 und von 40 bis 60°C zeigt;
  - iii) Feststellen, ob die genannte Xylanase eine Xylanase der Familie 11 ist und nach einem 30-minütigen Vorinkubations-Schritt bei 70°C, 80°C oder 90°C in der Gegenwart von 40% Glycerin, einem 30- oder 60-minütigen Vorinkubations-Schritt bei 62,5°C in Abwesenheit eines Stabilisators oder einem 30-minütigen Vorinkubations-Schritt bei 64°C oder 68°C in Abwesenheit eines Stabilisators mindestens 30% der optimalen Aktivität zeigt;
  - iv) Feststellen, ob die genannte Xylanase eine basische Aminosäure in Position 162 (*Trichoderma reesei* Xylanase 11 Numerierung), wobei diese Position durch einen Sequenzvergleich der genannten isolierten Xylanase mit der Aminosäuresequenz der genannten *Trichoderma reesei* Xylanase II, wie sie in SEQ ID NO:16 definiert ist, festgestellt wird, und eine intramolekulare Disulfid-Bindung besitzt, und
  - v) Gewinnen oder Zurückbehalten der Xylanase, die diese Eigenschaften zeigt.
11. Verfahren nach Anspruch 10, worin Schritt i) das teilweisen Reinigen der Xylanase umfasst.
12. Verfahren zum Herstellen von Tierfutter, umfassend das Aufbringen der isolierten Xylanase gemäß Anspruch 1 auf das Tierfutter, um eine Xylanase-Tierfutter-Kombination zu erzeugen, und Hitzesterilisieren der genannten Xylanase-Tierfutter-Kombination.
13. Verfahren gemäß Anspruch 12, worin das Tierfutter ein Geflügel- oder Schweinefutter ist.
14. Verfahren zum Herstellen von Tierfutter, umfassend das Aufbringen der aus Schritt v) des Anspruchs 10 erhaltenen Xylanase auf das genannte Tierfutter, um eine Xylanase-Tierfutter-Kombination zu erzeugen, und Hitzesterilisieren der genannten Xylanase-Tierfutter-Kombination.
15. Verfahren nach Anspruch 14, worin das Tierfutter ein Geflügel- oder Schweinefutter ist.
16. Isolierte Xylanase gemäß Anspruch 1, worin die Xylanase rekombinant ist.
17. Isolierte Xylanase gemäß Anspruch 1, umfassend eine (einzige) Disulfid-Brücke.
18. Isolierte Xylanase gemäß Anspruch 1, worin die Xylanase aus einem Organismus gewonnen wurde, der ausgewählt ist aus der aus *Aspergillus niger*, *Aspergillus kawachii*, *Aspergillus tubingensis*, *Bacillus circulans*, *Bacillus pumilus*, *Bacillus subtilis*, *Cellulomonas fimi*, *Chaetia* spp., *Clostridium acetobutylicum*, *Clostridium stercoarum*, *Fibrobacter succinogenes*, *Neocallimastix patriciarum*, *Nocardiopsis dassonvillei*, *Ruminococcus flavefaciens*, *Schizothraustes communis*, *Streptomyces lividans*, *Streptomyces* sp. Nr. 36a, *Streptomyces thermoviolaceus*, *Thermomonospora fusca*, *Trichoderma harzianum*, *Trichoderma reesei* und *Trichoderma viride* umfassenden Gruppe.
19. Isolierte Xylanase der Familie 11, dadurch gekennzeichnet, dass sie mindestens eine intramolekulare Disulfid-

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Brücke und eine basische Aminosäure in Position 162 (*Trichoderma reesei* Xylanase 11 Numerierung) oder deren Äquivalent umfasst, wobei diese Position durch Sequenzvergleich der genannten isolierten Xylanase mit der Aminosäuresequenz der genannten *Trichoderma reesei* Xylanase II, wie sie in SEQ ID NO:16 definiert ist, festgelegt wurde, wobei die genannte Xylanase, im Vergleich mit Xylanase vom Wildtyp, nach einem 30 minütigen Vorinkubations-Schritt bei 70°C, 80°C oder 90°C in der Gegenwart von 40% Glycerin, einem 30- oder 60 minütigen Vorinkubations-Schritt bei 62,5°C in Abwesenheit eines Stabilisators oder einem 30-minütigen Vorinkubations-Schritt bei 64°C oder 68°C in Abwesenheit eines Stabilisators mindestens 30% der optimalen Aktivität zeigt.

## Revendications

1. Xylanase isolée de la famille 11 comprenant au moins un pont disulfure intramoléculaire, et un acide aminé basique à la position 162 (numérotation de la xylanase II de *Trichoderma reesei*) ou son équivalent, ladite position étant déterminée à partir de l'alignement de séquence de ladite xylanase isolée avec ladite séquence d'acides aminés de la xylanase II de *Trichoderma reesei* définie dans SEQ ID N° : 16, ladite xylanase isolée présentant au moins 40 % d'activité optimale lorsqu'elle est comparée à la xylanase de type sauvage de pH 3,5 à pH 6,0, et de 40 à 60°C, et au moins 30 % d'activité optimale après une étape de pré-incubation de 30 minutes à 70°C, 80°C ou 90°C en présence de 40 % de glycérol ;  
 une étape de pré-incubation de 30 ou 60 minutes à 62,5°C en l'absence d'un stabilisateur ; ou  
 une étape de pré-incubation de 30 minutes à 64°C ou 68°C en l'absence d'un stabilisateur.
2. Xylanase isolée selon la revendication 1, dans laquelle ledit acide aminé basique est choisi dans le groupe constitué par la lysine, l'arginine et l'histidine.
3. Xylanase isolée selon la revendication 2, dans laquelle ledit acide aminé basique est l'histidine.
4. Xylanase isolée selon la revendication 1, comprenant deux ponts disulfure.
5. Xylanase isolée selon la revendication 1, choisie dans le groupe constitué par TrX-162H-DS1, TrX-162H-DS2, TrX-162H-DS4, et TrX-DS8.
6. Xylanase isolée selon la revendication 5, dans laquelle ladite xylanase est TrX-162H-DS1.
7. Xylanase isolée selon la revendication 5, dans laquelle ladite xylanase est TrX-162H-DS2.
8. Xylanase isolée selon la revendication 5, dans laquelle ladite xylanase est TrX-162H-DS4.
9. Xylanase isolée selon la revendication 5, dans laquelle ladite xylanase est TrX-DS8.
10. Procédé d'obtention d'une xylanase de la famille 11, comprenant :
  - i) la sélection d'un organisme qui exprime l'activité xylanase, et l'obtention de ladite xylanase dudit organisme ;
  - ii) la détermination du fait que la xylanase présente ou non au moins 40 % d'activité optimale lorsqu'elle est comparée à la xylanase de type sauvage de pH 3,5 à pH 6,0, et de 40 à 60°C ; et
  - iii) la détermination du fait que ladite xylanase est une xylanase de la famille 11 et présente au moins 30 % d'activité optimale après  
 une étape de pré-incubation de 30 minutes à 70°C, 80°C ou 90°C en présence de 40 % de glycérol ;  
 une étape de pré-incubation de 30 ou 60 minutes à 62,5°C en l'absence d'un stabilisateur ; ou  
 une étape de pré-incubation de 30 minutes à 64°C ou 68°C en l'absence d'un stabilisateur ;
  - iv) la détermination du fait que ladite xylanase a ou non un acide aminé basique à la position 162 (numérotation de la xylanase II de *Trichoderma reesei*), ladite position étant déterminée à partir de l'alignement de séquence de ladite xylanase isolée avec ladite séquence d'acides aminés de la xylanase II de *Trichoderma reesei* définie dans SEQ ID N° : 16, et un pont disulfure intramoléculaire ; et
  - v) la conservation de ladite xylanase qui exprime ces propriétés.
11. Procédé selon la revendication 10, dans lequel l'étape i) comprend la purification partielle de ladite xylanase.

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12. Procédé de préparation de nourriture pour animaux comprenant l'application de la xylanase isolée selon la revendication 1 sur ladite nourriture pour animaux pour produire une combinaison de xylanase-nourriture pour animaux, et la stérilisation thermique de ladite combinaison de xylanase-nourriture pour animaux.

5 13. Procédé selon la revendication 12, dans lequel ladite nourriture pour animaux est de la nourriture pour la volaille ou les porcs.

14. Procédé de préparation de nourriture pour animaux comprenant l'application de la xylanase obtenue dans l'étape v) de la revendication 10 sur ladite nourriture pour animaux pour produire une combinaison xylanase-nourriture pour animaux, et la stérilisation thermique de ladite combinaison de xylanase-nourriture pour animaux.

15. Procédé selon la revendication 14, dans lequel ladite nourriture pour animaux est de la nourriture pour la volaille ou les porcs.

16. Xylanase isolée selon la revendication 1, dans laquelle la xylanase est recombinante.

17. xylanase isolée selon la revendication 1, comprenant un pont disulfure.

18. Xylanase isolée selon la revendication 1, ladite xylanase étant obtenue à partir d'un organisme choisi dans le groupe constitué par *Aspergillus niger*, *Aspergillus kawachii*, *Aspergillus tubingensis*, *Bacillus circulans*, *Bacillus pumilus*, *Bacillus subtilis*, *Cellulomonas fimi*, *Chelonia spp.*, *Clostridium acetobutylicum*, *Clostridium stercorarium*, *Fibrobacter succinogenes*, *Neocallimastix patriciarum*, *Nocardia sp.*, *Ruminococcus flavefaciens*, *Schizophyllum commune*, *Streptomyces lividans*, *Streptomyces sp. No. 38a*, *Streptomyces thermoviolaceus*, *Thermomonospora fusca*, *Trichoderma harzianum*, *Trichoderma reesei*, et *Trichoderma viride*.

19. Xylanase isolée de la famille 11 caractérisée en ce qu'elle comprend au moins un pont disulfure intramoléculaire, et un acide aminé basique à la position 182 (numérotation de la xylanase II de *Trichoderma reesei*) ou son équivalent, ladite position étant déterminée à partir de l'alignement de séquence de ladite xylanase isolée avec ladite séquence d'acides aminés de la xylanase II de *Trichoderma reesei* définie dans SEQ ID N° : 16, ladite xylanase isolée présentant au moins 30 % d'activité optimale lorsqu'elle est comparée à la xylanase de type sauvage après une étape de pré-incubation de 30 minutes à 70°C, 80°C ou 90°C en présence de 40 % de glycérol ; une étape de pré-incubation de 30 ou 60 minutes à 62,5°C en l'absence d'un stabilisateur ; ou une étape de pré-incubation de 30 minutes à 64°C ou 68°C en l'absence d'un stabilisateur.

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				S	AFNTQAAP	31
				G		1
Ca	23					
Cs	1					
Bp	1	RTITNNEMGN	HSGYDYELWK DYGNT-SMTL NNGGAFSAGW N--NIGNA			45
Ca	32	KTITSNEIGV	NGGYDYELWK DYGNT-SMTL KNGGAFSCQW S--NIGNA			76
Cs	2	RIIYDNETGT	HGGYDYELWK DYGNT-IMEL NDGGTFSCQW S--NIGNA			46
Rf	1	SAADQOTRGN	VGGYDYEMWN QNGGQASMN PGAGSFTCSW S--NIENF			46
Tr2	1	QTIQPGTGY	NNGYFYSYWN DGHGGVITYN GPGGQFSVWN S--NSGNF			45
Tv	1	QTIQPGTGF	NNGYFYSYWN DGHGGVITYN GPGGQFSVWN S--NSGNF			45
Th	1	QTIQPGTGY	SNGYFYSYWN DGHAGVITYN GGGGSFTVWN S--NSGNF			45
Sc	1	SGTPSSTGT	DGGYYSWWT DGAGDATYON NGGGSYTLTH SG--NNGNL			46
An	1		AGINYVQNYN GNLGDFTY-D ESAGTFSMYH EDGVSSDF			38
AT	1		AGINYVQNYN QNLGDFTY-D ESAGTFSMYH EDGVSSDF			38
Tr1	1		ASINYDQNYQ TGG-QVSYN PSNTGFSVWN N--TQDDF			34
Ss	1	ATTIT-NETGY	D-GMYSPWT DGGGSVSMTL NCGGSYSTRN T--NCGNF			45
SlB	1	DTVVITNQEGT	MNGYYSFWT DSQGTVMNM CSQQYSTSW R--NTGNF			47
SlC	1	ATTITNQGT	D-GMYSPWT DGGGSVSMTL NCGGSYSTQN T--NCGNF			46
Tf	1	AVTSNETGY	HDGYYSFWT DAPGTVMEL GPGGNYSVWN R--NTGNF			45
Bc	1		ASTDYNQWNT DGGGIVNAVN GSGGNYSVWN S--NTGNF			36
Bs	1		ASTDYNQWNT DGGGIVNAVN GSGGNYSVWN S--NTGNF			36
Bp	46	LFRK-GKKFD	ST-RTHQLG NISINYNASF N-PSGNLYC VYGHQSP			90
Ca	77	LFRK-GKKFN	DT-QTYQLG NISVNYNCNY Q-PYGNLYC VYGHQSP			121
Cs	47	LFRK-GKKFN	SD-KTYQLG DIVVEYCCDY N-PNGNSYL C VYGHTRNP			91
Rf	47	LARM-GKNYD	SQKQYKAFG NIVLTYDVEY T-PRGNLYC VYGHTRNP			92
Tr2	46	VGGK-GWQPG	TKNKV-----INFS-GS YNPNGNSYLS VYGHTRNP			83
Tv	46	VGGK-GWQPG	TKNKV-----INFS-GS YNPNGNSYLS VYGHTRNP			83
Th	46	VGGK-GWQPG	TKNKV-----INFS-GS YNPNGNSYLS VYGHTRNP			83
Sc	47	VGGK-GWQPG	AASRS-----ISYS-GT YQPNNGSYLS VYGHTRNP			84
An	39	VVGL-GWTTG	SSNA-----ITYSABY SASGSSSYLA VYGHVNYN			76
At	39	VVGL-GWTTG	SSNA-----ITYSABY SASGSSSYLA VYGHVNYN			77
Tr1	35	VVGK-GWTTG	SSAP-----INFGGSP SVN5GTCLLS VYGHSTNP			72
Ss	46	VAGK-GWANG	GR-RT-----VRYT-GW FNPNGNGYGC LYGHSTNP			82
SlB	48	VAGK-GWANG	GR-RT-----VOYS-GS FNPNGNAYLA LYGHSTNP			84
SlC	47	VAGK-GWATG	DGN-----VRYN-GY FNPVNGYGC LYGHSTNP			82
Tf	46	VAGK-GWATG	GR-RT-----VTYS-AS FNPNGNAYLT LYGHTRNP			82
Bc	37	VVGK-GWTTG	SPFRT-----INYNAGV WAPNGNGYLT LYGHTRSP			75
Bs	37	VVGK-GWTTG	SPFRT-----INYNAGV WAPNGNGYLT LYGHTRSP			75

FIGURE 1

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Bp 91 LAEYIVDSW GTYR-PT--G AYKGSFYADG GTYDIYETTR VNQPSIIG 135  
 Ca 122 LVEYVIDSW GSWRPP--GG TSKGTITVDG GIYDIYETTR INQPSIQG 167  
 Cs 92 LVEYVIVESW GSWRPP--GA TPKGTITQWMAETIYETTR VNQPSIDG 138  
 Rf 93 LMEYIVEGW GDWRPPGNDG EVKGTVSANG NTYDIRKTR VNQPSIDG 140  
 Tr2 84 LIEYIVENF GTYN-PSTGA TKLGEVTSDE SVYDIYRTOR VNQPSIIG 130  
 Tv 84 LIEYIVENF GTYN-PSTGA TKLGEVTSDE SVYDIYRTOR VNQPSIIG 130  
 Th 84 LIEYIVENF GTYN-PSTGA TKLGEVTSDE SVYDIYRTOR VNQPSIIG 130  
 Sc 85 LIEYIVESY GSYD-PSSAA SHKGSVTCNG ATYDILSTWR VNEPSIDG 131  
 An 77 GAEYIVEDY GDYN-PCSSA TSLGTVYSDG STYQVCTDTR INEPSITG 123  
 At 78 GAEYIVEDY GDYN-PCSSA TSLGTVYSDG STYQVCTDTR INEPSITG 124  
 Tr1 73 LVEYIMEON HNY--PAQ-G TVKGTVTSDE ATYTIWENTR VNEPSIQG 117  
 Ss 83 LVEYIVDNW GSYR-PT--G ETRGTVHSDG GTYDIYKTR VNEPSVEG 127  
 SlB 85 LVEYIVDNW GTYR-PT--G EYKGTVTSDE GTYDIYKTR VNEPSVEG 129  
 SlC 83 LVEYIVDNW GSYR-PT--G TYKGTVSSDG GTYDIYQTR VNEPSIEG 127  
 Tf 83 LVEYVIVESW GTYR-PT--G TYMGTVTIDG GTYDIYKTR VNEPSIEG 127  
 Bc 76 LIEYVVDSW GTYR-PT--G TYKGTVKSDE GTYDIYTTTR VNEPSIDG 120  
 Bs 76 LIEYVVDSW GTYR-PT--G TYKGTVKSDE GTYDIYTTTR VNEPSIDG 120

Bp 136 -IATEKQYWE VRQTKRTS-- -----GTVS VSAHFRKYES LQMPM-GK 174  
 Ca 168 -NTTEKQYWE VRTKRTS-- -----GTIS VSKHPAAHES KQMLP-GK 206  
 Cs 139 -TATEQQYWE VRTSKRTS-- -----GTIS VTEHFKQNER MGRM-GK 177  
 Rf 141 -TATEPQYWE VRQTSQSANN QTNMGTID VSKHFDANSA AGLDMSGT 187  
 Tr2 131 -TATEYQYWE VRNRHR-S-S -----GSVN TANHFNAHAQ QGLTL-GT 168  
 Tv 131 -TATEYQYWE VRNRHR-S-S -----GSVN TANHFNAHAQ QGLTL-GT 168  
 Th 131 -TATEYQYWE VRNRHR-S-S -----GSVN TANHFNAHAQ QGLTL-GT 168  
 Sc 132 -TQTEQYWE VRNPKAPGG SIS---GTVD VOCHFDANKG LQNLGSE 175  
 An 124 -TSTETQYFE VRESTRTS-- -----GTVT VANHFNFNAH HGFEN-SD 163  
 At 125 -TSTETQYFE VRESTRTS-- -----GTVT VQNHFN-WAS LGLHLGQM 155  
 Tr1 118 -TATEQYIS VRNSPR-T-S -----GTIT TGNHFDANAR AGMNMGNF 168  
 Ss 128 -PAAEQYWE VRQSKVT--S -----GTIT TGNHFDANAR AGMPLGNF 168  
 SlB 130 TR-TEDQYWE VRQSKR-TG- -----GTIT TGNHFDANAR AGNMGOQ 168  
 SlC 128 TK-TEDQYWE VRQSKVTSGS -----GTIT TGNHFDANAR AGMHLGTH 166  
 Tf 128 TR-TEDQYWE VRQSKRTS-- -----GTIT TGNHFDANAR AGMHLGTH 166  
 Bc 121 DRTTETQYWE VRQSKRPTGS N-----ATIT FSNHVNANKS HGMNLGSN 163  
 Bs 121 DRTTETQYWE VRQSKRPTGS N-----ATIT FSNHVNANKS HGMNLGSN 163

FIGURE 1 (CONT'D)

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Bp	174	MYETAFTVEG	YQSSGSANVM	TNQLFIGN	201
Ca	207	MHETAFNIEG	YQSSGKADVN	SMSINICK	233
Cs	178	MYEVALTVEG	YQSSGYANVY	KNEIRIGANP....	
Rf	188	LYEVSLNIEG	YRSNGSANVK	SVSV	211
Tr2	169	MDYQIVAVEG	YFSSGSASI-	TVS	190
Tv	169	MDYQIVAVEG	YFSSGSASI-	TVS	190
Th	169	MDYQIVAVEG	YFSSGSASI-	TVS	190
Sc	176	HNYQIVATEG	YQSSGTATI-	TVT	197
An	163	FNYQVMAVEA	WCGAGSASV-	TISS	184
At	164	FNYQVMAVEA	WCGAGSAAV-	TISS	185
Tr1	156	MNYQVVAVEG	WCGSGSASQ-	SVSN	178
Ss	167	RYYMINATEG	YQSSGSSTI-	TVSG	189
SlB	169	SYMINATEG	YQSSGTSSI-	NVGG.....	
SlC	169	RYYMINATEG	YQSSGSSNI-	TVSG	191
Tf	167	D-YMIMATEG	YQSSGSSNVT	LGTS.....	
Bc	164	WAYQVMATEG	YQSSGSSNV-	TVW	185
Bs	164	WAYQVMATEG	YQSSGSSNV-	TVW	185

Bp	<i>Bacillus pumilus</i>
Ca	<i>Clostridium acetobutylicum</i> P262 XynB
Cs	<i>Clostridium stercorarium</i> xynA
Rf	<i>Ruminococcus flavefaciens</i>
Tr2	<i>Trichoderma reesei</i> XYN II
Tv	<i>Trichoderma viride</i>
Th	<i>Trichoderma harzianum</i>
Sc	<i>Schizophyllum commune</i> Xylanase A
An	<i>Aspergillus niger</i> , var. <i>awamori</i>
At	<i>Aspergillus tubigenis</i>
Tr1	<i>Trichoderma reesei</i> XYN I
Ss	<i>Streptomyces</i> sp. 36a
SlB	<i>Streptomyces lividans</i> Xln B
SlC	<i>Streptomyces lividans</i> Xln C
Tf	<i>Thermomonospora fusca</i> TfxA
Bc	<i>Bacillus circulans</i>
Bs	<i>Bacillus subtilis</i>

FIGURE 1 (CONT'D)



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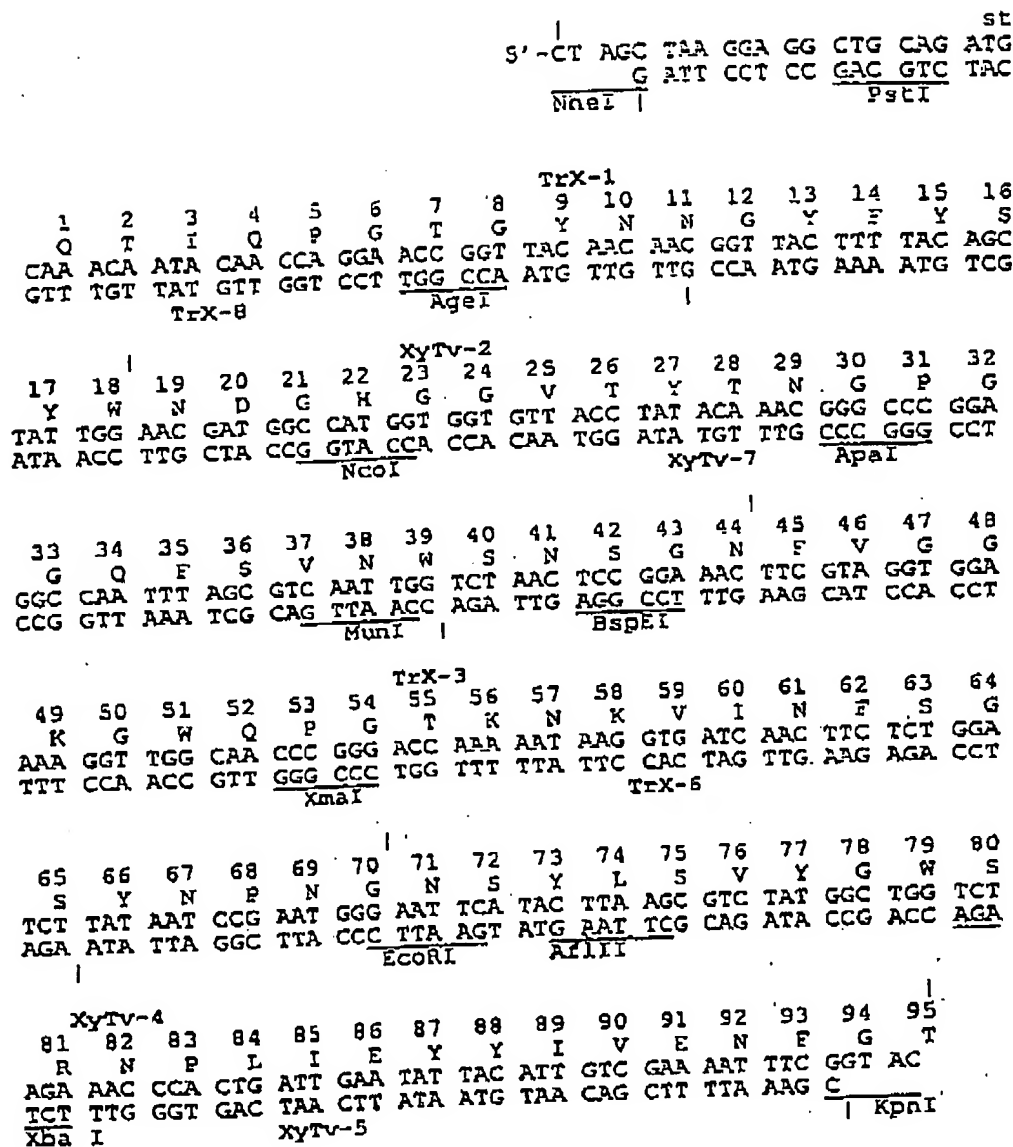


FIG. 2

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1  
 V D 92 93 94 95 96 97 98 99 100 101 102 103 104 105  
 TC GAC AAT TTC GGT ACC TAC AAT CCG AGT ACC GGC GCC ACA AAA TTA  
 G TTA AAG CCA TGG ATG TTA GGC TCA TGG CCG CGG TGT TTT AAT  
 SalI | KpnI XyTV-101 XyTV-110 Kasi/Nari

106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121  
 G E V T S D G S V Y D I Y R T Q  
 GGC GAA GTC ACT AGT GAT GGA TCC GTA TAT GAT ATC TAC CGT ACC CAA  
 CCG CTT CAG TGA TCA CTA CCT AGG CAT ATA CTA TAG ATG GCA TGG GTT  
 SpeI | BamHI XyTV-102 XyTV-109

122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137  
 R V N Q P S I I G T A T F Y Q Y  
 CGC GTT AAT CAG CCA TCG ATC ATT GGA ACC GCC ACC TTT TAT CAG TAC  
 CCG CAA TTA GTC GGT AGC TAG TAA CCT TGG CCG TGG AAA ATA GTC ATG  
 MluI | ClaI Trx-103

138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153  
 W S V R N H R S G S V N T A  
 TGG AGT GTT AGA CGT AAT CAT CGG AGC TCC GGT TCG GTT AAT ACT GCG  
 ACC TCA CAA TCT GCA TTA GTA GCC TCG AGG CCA AGC CAA TTA TGA CGC  
 Trx-108 SacI

154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169  
 N H F N A W A Q Q G L T L G T M  
 AAT CAC TTT AAT GCA TGG GCA CAG CAA GGG TTA ACC CTA GGT ACA ATG  
 TTA GTG AAA TTA CGT ACC CGT GTC GTT CCC AAT TGG GAT CCA TGT TAC  
 NsiI | XyTV-104 XyTV-107 AvrII

170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185  
 D Y Q I V A V E G Y F S S G S A  
 GAT TAT CAA ATC GTA GCG GTG GAA GGC TAC TTC TCG AGT GGT TCC GCT  
 CTA ATA GTT TAG CAT CCG CAC CTT CCG ATG AAG AGC TCA CCA AGG CGA  
 XyTV-105 XyTV-106 XhoI

186 187 188 189 190  
 S I T V S  
 AGT ATT ACA GTG AGC TAA A  
 TCA TAA TGT CAC TCG ATT TCT AG  
 BglII |

FIG. 2 (CONT'D)

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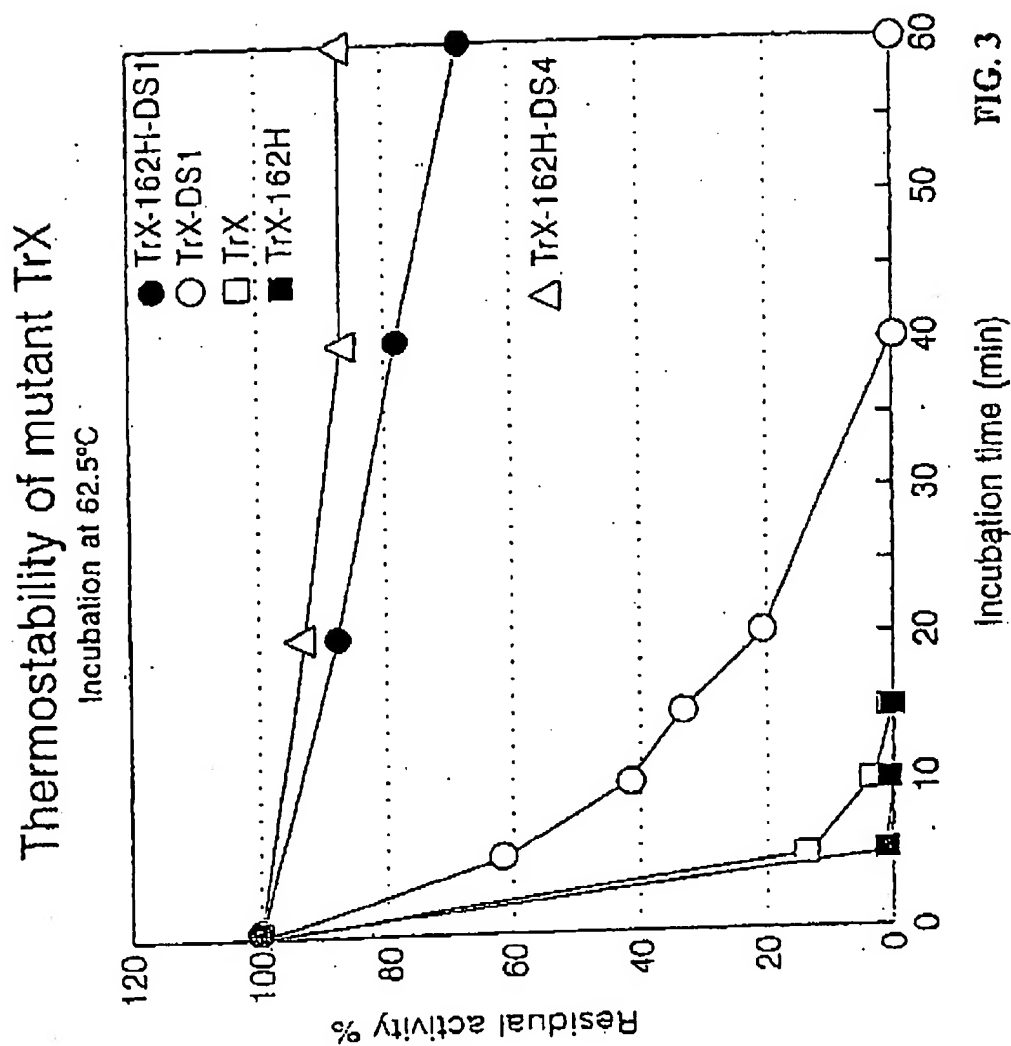


FIG. 3

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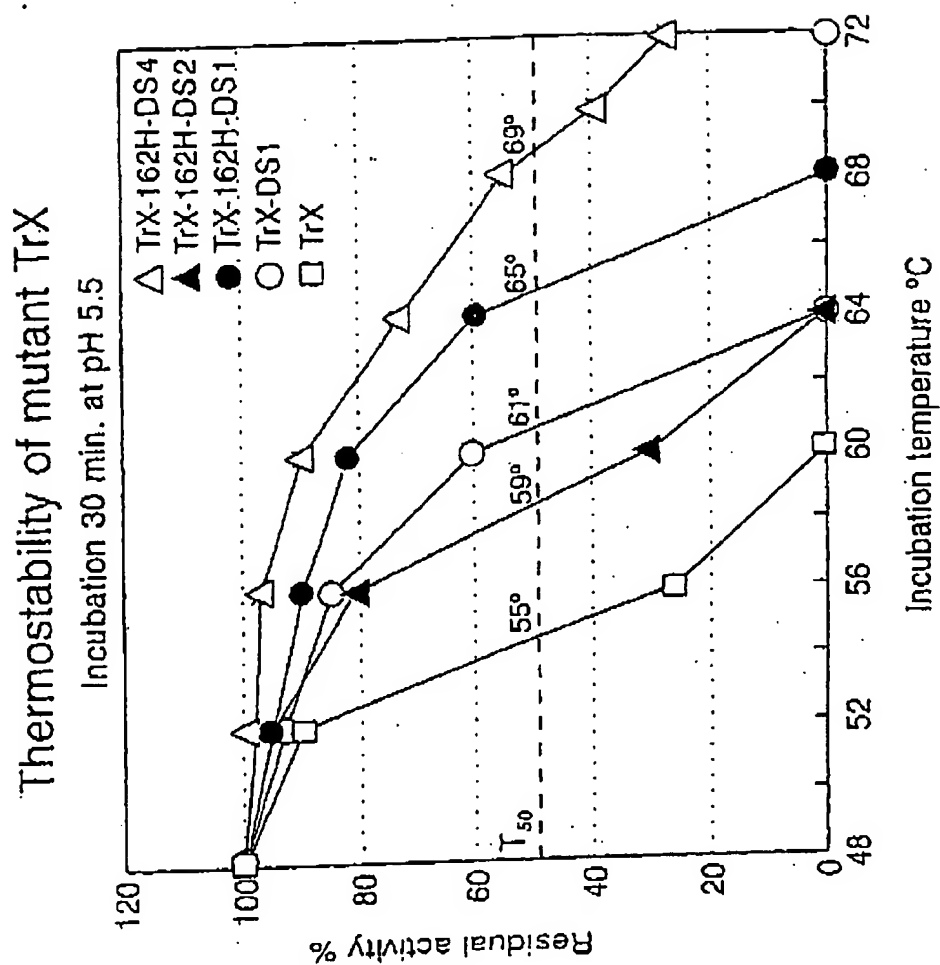
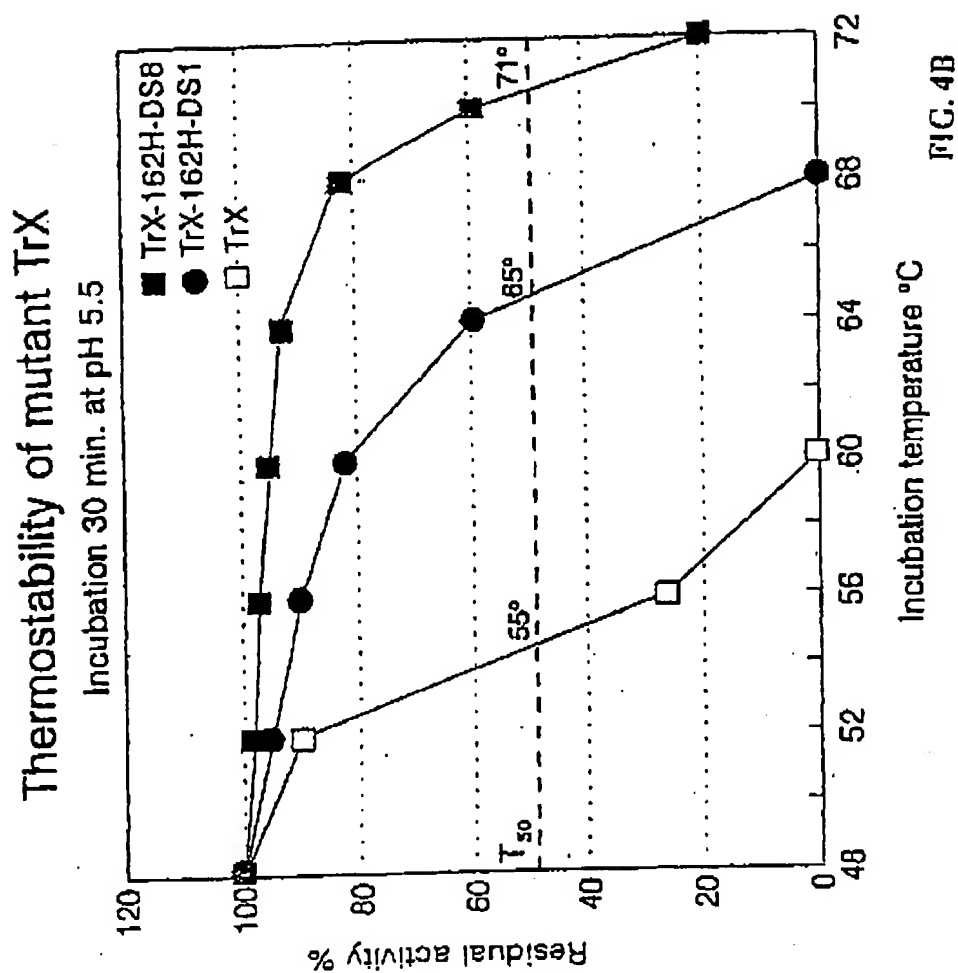


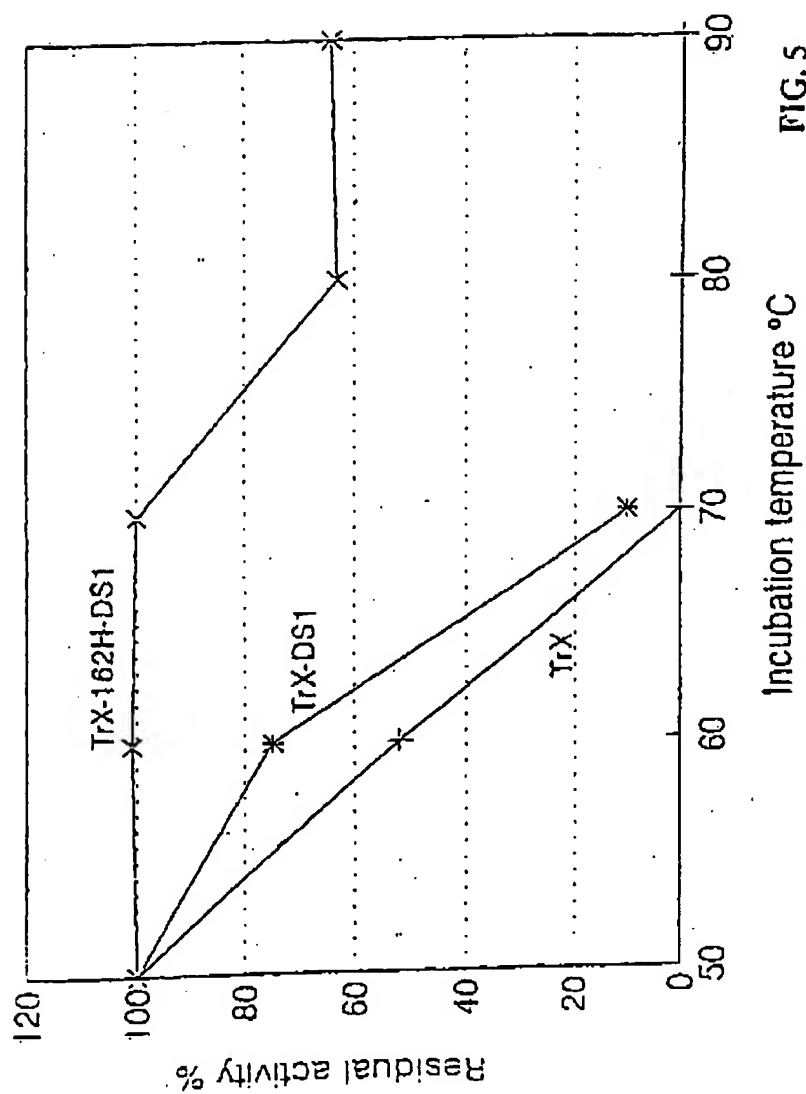
FIG. 4A

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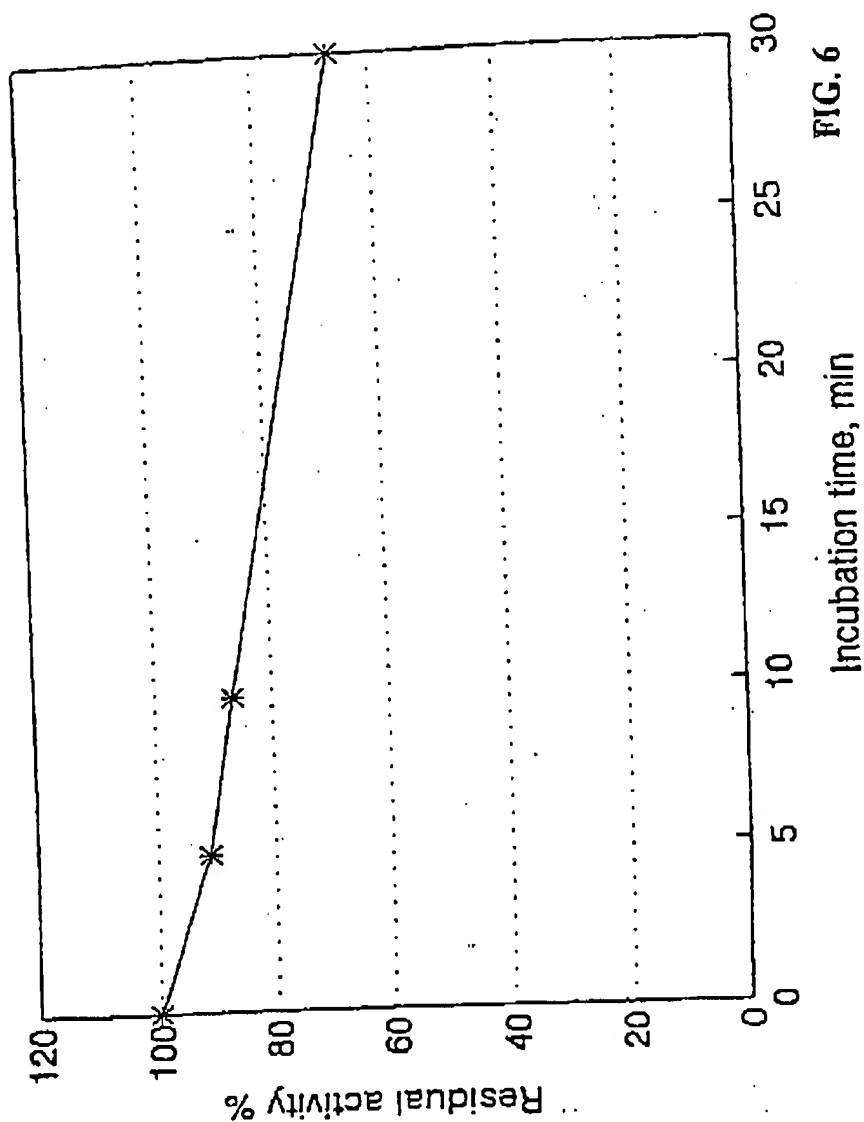
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Thermostability of mutant TrX  
incubation in 40% glycerol, 30 min



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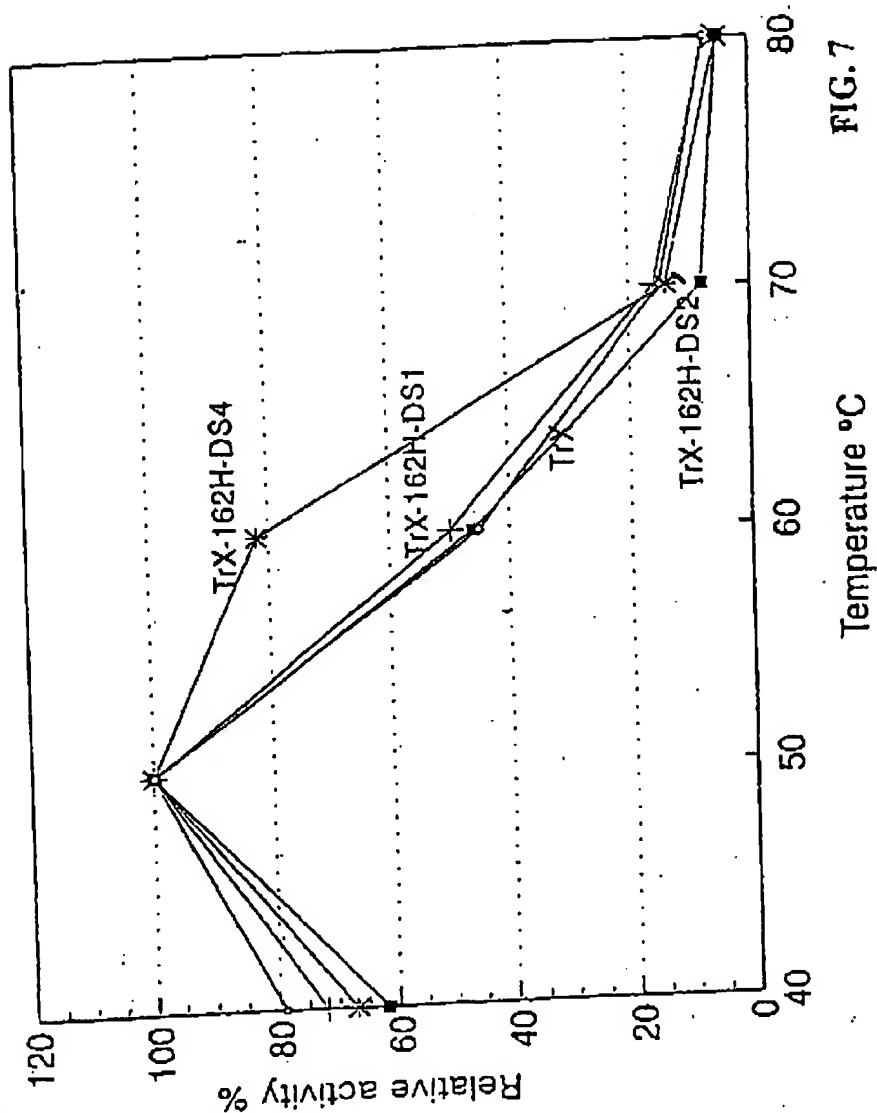
## Thermostability of TrX-162H-DS1 at 90°C



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# Effect of temperature on the hydrolysis of xylan by mutant TrX

pH 4.5, 30 min, 1.5 microgram of each enzyme





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